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ORIGINAL RESEARCH

Identification and transcription profiling of *NDUFS8* in *Aedes taeniorhynchus* (Diptera: Culicidae): developmental regulation and environmental response

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Abstract: The cDNA of a NADH dehydrogenase-ubiquinone Fe-S protein 8 subunit (*NDUFS8*) gene from *Aedes (Ochlerotatus) taeniorhynchus* Wiedemann has been cloned and sequenced. The 824 bp full-length mRNA sequence of *AetNDUFS8* encodes an open reading region of 651 bp (that is, 217 amino acids). To identify if *AetNDUFS8* is developmentally regulated, we employed a quantitative real-time polymerase chain reaction to examine *AetNDUFS8* gene expression levels in all developmental stages of *Ae. taeniorhynchus*. In egg, larval, and pupal stages, *AetNDUFS8* was expressed at relatively low levels. However, in the teneral adult stage, *AetNDUFS8* was highly expressed. During the time course study in response to permethrin pesticide treatment, quantitative real-time polymerase chain reaction (PCR) also showed that mRNA transcription levels of *AetNDUFS8* were regulated in female *Ae. taeniorhynchus*. A mitochondrially encoded NADH dehydrogenase subunit 5 *AetNADH5* was highly expressed in different developmental stages of *Ae. taeniorhynchus*. This study suggests that *AetNDUFS8* and *AetNADH5* play an essential role in the development of *Ae. taeniorhynchus* and will provide information useful for developing dsRNA pesticide for mosquito control.

Keywords: *Aedes taeniorhynchus*, *AetNDUFS8*, mRNA expression, development, permethrin

Introduction

NADH dehydrogenase, located in the inner mitochondrial membrane, is the first enzyme of the mitochondrial electron transport chain that catalyzes the transfer of electrons from NADH to coenzyme Q.[1,2] Mitochondrial genes or mitochondrial related genes can be used as genetic markers to identify ecotypes in different populations of plants,[3,4] and animals, including insects and mosquitoes.[5–13] The critical role of NADH in the respiratory function of Complex I has been demonstrated by linking mutation in NADH subunits to certain hereditary disease, such as disease-causing mtDNA-encoded ND6 gene mutation.[14]

Aedes taeniorhynchus Wiedemann, a nuisance species, has attracted much attention recently.[9,15–20] The aim of this study was to clone the NADH dehydrogenase-ubiquinone Iron-Sulfate (Fe-S) protein 8 subunit (*AetNDUFS8*) gene and examine mRNA expression during development and in response to challenge by the permethrin pesticide.[21] This information is important for understanding the role of NADH subunits in development and pesticide sensitivity in mosquitoes. Mitochondrially encoded NADH dehydrogenase subunit 5 (*AetNADH5*) has been

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14. ABSTRACT

The cDNA of a NADH dehydrogenase-ubiquinone Fe-S protein 8 subunit (NDUFS8) gene from Aedes (Ochlerotatus) taeniorhynchus Wiedemann has been cloned and sequenced. The 824 bp full-length mRNA sequence of AetNDUFS8 encodes an open reading region of 651 bp (that is, 217 amino acids). To identify if AetNDUFS8 is developmentally regulated, we employed a quantitative real-time polymerase chain reaction to examine AetNDUFS8 gene expression levels in all developmental stages of Ae. taeniorhynchus. In egg, larval, and pupal stages, AetNDUFS8 was expressed at relatively low levels. However, in the teneral adult stage, AetNDUFS8 was highly expressed. During the time course study in response to permethrin pesticide treatment, quantitative real-time polymerase chain reaction (PCR) also showed that mRNA transcription levels of AetNDUFS8 were regulated in female Ae. taeniorhynchus. A mitochondrially encoded NADH dehydrogenase subunit 5 AetNADH5 was highly expressed in different developmental stages of Ae. taeniorhynchus. This study suggests that AetNDUFS8 and AetNADH5 play an essential role in the development of Ae. taeniorhynchus and will provide information useful for developing dsRNA pesticide for mosquito control.

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used to identify the species on isolated oceanic islands.[9] In the present study, *AetNADH5* was examined during the development of *Ae. taeniorhynchus*. As part of our effort to develop new toxicants for applied mosquito control, understanding the role of mitochondrial genes from *Ae. taeniorhynchus* in development and pesticide exposure may provide the information needed to identify and develop novel mosquito control strategies.[22] Applying RNA interference (RNAi) technology to silence the mitochondrial proteins, *AetNDUFS8* and *AetNADH5* genes may provide additional targets for developing dsRNA pesticides.[22,23]

Materials and methods

Mosquito strain

Ae. taeniorhynchus (Orlando, Florida strain, maintained since 1952) was reared in the insectary of the Mosquito and Fly Research Unit at the Center for Medical, Agricultural, and Veterinary Entomology, Agriculture Research Service, United States Department of Agriculture, Gainesville, FL. Temperature was maintained at 27°C and humidity, 80% RH, respectively. Adult females without blood feed were maintained on 5% sucrose during all experiments.[24]

At each larval time point, samples were collected, each containing 100–150 larvae. Three samples were frozen in liquid nitrogen and then stored in the −80°C freezer for RNA isolation. The remainder were fixed in 90% ethanol for measuring. The transverse diameter of head capsules was measured with a dissecting microscope (model Stemi SV8, Carl Zeiss, Thomwood, NY, USA) connected to a camera (model 11.2 Color Mosaic, Diagnostic Instruments, Sterling Heights, MI, USA).

Instar status was determined by the transverse diameter of the head capsules; 1st instar (mean, 0.319±0.034 mm), 2nd instar (0.444±0.092 mm), 3rd instar (0.731±0.155 mm), and 4th instar (1.057±0.159 mm).

RNA extraction

All developmental stages (ie, eggs, larvae, pupae, and adults) of *Ae. taeniorhynchus* were collected at various time points within each stage. Fifty micrograms of three samples for the egg stage were collected. About 100–200 larvae for the larval stage were collected for the RNA extraction. About 15–20 pupae and adults were assembled for each sample. Three replicates of the experiment were conducted. To the samples 1 mL TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was added and ground with tissue miser homogenizer. The total RNAs were then extracted according to the manufacturer's instructions. RNA samples were quantitated using the NanoDrop 2000, UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA).

GeneRacer cloning and gene sequencing of GeneRacer library

GeneRacer cloning and gene sequencing of GeneRacer library were described in a previous publication.[24] Selected plasmids were sequenced at the Sanger Sequencing Core Laboratory in the Interdisciplinary Center for Biotechnology Research, University of Florida (Gainesville, FL, USA). The DNA sequence was analyzed using SDSC Biology Workbench – San Diego Supercomputer Center (http://workbench.sdsc.edu) and ExPASy (http://www.expasy.org/). The sequence was submitted to National Center for Biotechnology Information; GenBank Accession number: FJ458415.

Permethrin experiments

Five-day old adult female *Ae. taeniorhynchus* were topically treated to the scutum of thorax with permethrin/acetone at 2.5×10^{-5} µg (high dose, HD) and 1.25×10^{-5} µg (low dose, LD) per mosquito as previously described by Zhao et al.[24,25] Ten mosquitoes per cup were used for all treatments.[24] At each time course point, including 0 (blank control), 5, 15, 30, 60, and 180 minutes, and 6 hours and 24 hours, we collected 30 females after permethrin pesticide treatment. A control group was topically exposed to acetone only.[25]

Quantitative real-time PCR amplification

The method of cDNA synthesis is the same as described in the previous publication.[24]

The quantitative real-time PCR (qPCR) assay for *AetNDUFS8* and *AetNADH5* mRNA expression in *Ae. taeniorhynchus* was performed as described in the previous publication.[24,26] The PCR primers for ribosomal 40s gene are AET-40S-52F (5′-TGATGAGGCTTCTCCCTACG-3′) and AET-40S-261R (5′-GGGATTGGGGTAACATCCTC-3′). The PCR primers for ribosomal 60s gene (GenBank accession number: FJ444827) are AET-60S-117F (5′-TCTGCGTAAGCGGTGTAATG-3′) and AET-60S-336R (5′-GGGTGGTATGCCTTCGTAGA-3′). The PCR primers used were AET-*NDUFS8*-141F (5′-CAAGGACCCCAGTATGGAGA-3′) and AET-*NDUFS8*-415R (5′-GCTCTTCCGCTTCTATGGTG-3′). The PCR primers (GenBank accession number: FM992318)

used were AET-NADH5-162F (5′-TCCAGAAATAATTTG TTTACCATTTT-3′) and AET-NADH5-425R (5′-CCTCCA AAATATTCACTTCAACC-3′). The PCR thermal cycling parameters were also the same as previously published.[24,27] Relative expression levels were determined as follows: first, *AetNADH* transcript levels relative to a standard (ribosomal 40s/60s) were obtained by applying the formula ΔCT = CT (*AetNADH*) – CT (*Aet*-ribosomal 40s/60s). Second, an average ΔCT value for each sample was determined. Third, relative expression levels were evaluated applying the modified equation $100\times2^{-[\text{average }\Delta CT]}$.[28,29]

Sequence data analysis

A multiple sequence alignment of *AetNDUFS8* and *AetNADH5* as well as orthologs from other mosquitoes were performed with the MEGA 5.05 program (http://www.megasoftware.net). The Neighbor-joining method with the MEGA 5.05 program was used to construct the phylogenetic trees.[30] The Neighbor-joining is a bottom-up clustering method for the creation of phenetic trees, based on the minimum-evolution criterion.[31]

Statistical analysis

To determine significant difference, the SigmaPlot software was used for comparing two groups of data (SigmaPlot®11.2, Inc., San Jose, CA, USA).

Results

Identification of *Ae. taeniorhynchus AetNDUFS8* gene

The full-length cDNA sequence of *AetNDUFS8* of *Ae. taeniorhynchus* was first deposited in GenBank by Zhao et al[24] (accession number FJ458415). The *AetNDUFS8* is 650 bp that codes for a protein of 217 amino acids with a molecular mass of 24.5 kDa (GenBank accession number: ACL37997). A comparison *AetNDUFS8* with *NDUFS8* in *Ae. aegypti* (L.) (AY432654.1), *Armigeres subalbatus* (Coquillett) (AY440457.1), *Anopheles gambiae* Giles (BX042513.1), *Culex quinquefasciatus* Say (XM 001868794.1), and *Drosophila* species (AC010122.7; XM_002016993.1; XM_001980072.1; XM_002030974.1; XM_001359220.2; AY070919.1; NM_079980.2) revealed that they share 84%, 83%, 82%, 81%, and 77%–82% identity, respectively. Using the Neighbor-joining method with MEGA 5.05 program, a phylogenetic tree for *AetNDUFS8* nucleic acid sequences from other insect orthologs was constructed (Figure 1). The phylogenetic analysis demonstrated that *AetNDUFS8* was closely related to *Armigeres subalbatus* Theobald and *Aedes aegypti* L. as well as *Anopheles gambiae* Giles.

AetNDUFS8 gene expression in all developmental stages of *Ae. taeniorhynchus*

To better understand how nuclear encoded mitochondrial genes are expressed during the development of *Ae. taeniorhynchus*, we inspected *AetNDUFS8* mRNA relative expression levels in all developmental stages (ie, eggs, larvae, pupae, and adults) employing qPCR (Figure 2, Tables S1–S3). In the egg stage, the relative mRNA expression level of *AetNDUFS8* slightly increased from day 1 to day 3 during the time course study. The mRNA relative expression level of *AetNDUFS8* was slightly increased through the development of the 1st instar larva in the early (5 hours posthatch) to the late (53 hours posthatch) stage samples. *AetNDUFS8* expression was relatively high in the 2nd instar larvae, the 3rd instar larvae, and early 4th instar larvae examined (from 69 hours posthatch to 125 hours posthatch). However, RNA relative expression level of *AetNDUFS8* was significantly higher in teneral male adults (mean, 33.158±1.573) when compared to teneral female adults (mean, 13.904±1.469). RNA expression of *AetNDUFS8* in the teneral male is significantly different from that in the teneral female (Table S2). mRNA expression levels of *AetNDUFS8* in teneral male/female *Ae. taeniorhynchus* were significantly higher than those found in 5- and 10-day-old adults (Figure 2, Tables S1 and S2).

AetNDUFS8 gene expression in response to permethrin

To examine if the mRNA transcription of *AetNDUFS8* in *Ae. taeniorhynchus* was affected by permethrin pesticide, 5-day old female mosquitoes were treated with two concentrations of permethrin (as described in the Materials and methods section) using acetone as a carrier. In female *Ae. taeniorhynchus,* the qPCR time courses of *AetNDUFS8* mRNA expressed differently between HD and LD of the permethrin. In the 5-day old female *Ae. taeniorhynchus*, *AetNDUFS8* expression decreased slightly for LD at 5 minutes post permethrin treatment and then increased for both doses at 15 minutes post permethrin treatment compared to treatment with acetone only as a control (Figure 3, Table S4, and S5). HD-treated and LD-treated permethrin/acetone for *Ae. taeniorhynchus* female adults showed a decrease in *AetNDUFS8* mRNA expression at 30 minutes postexposure, but a significant increase in *AetNDUFS8*

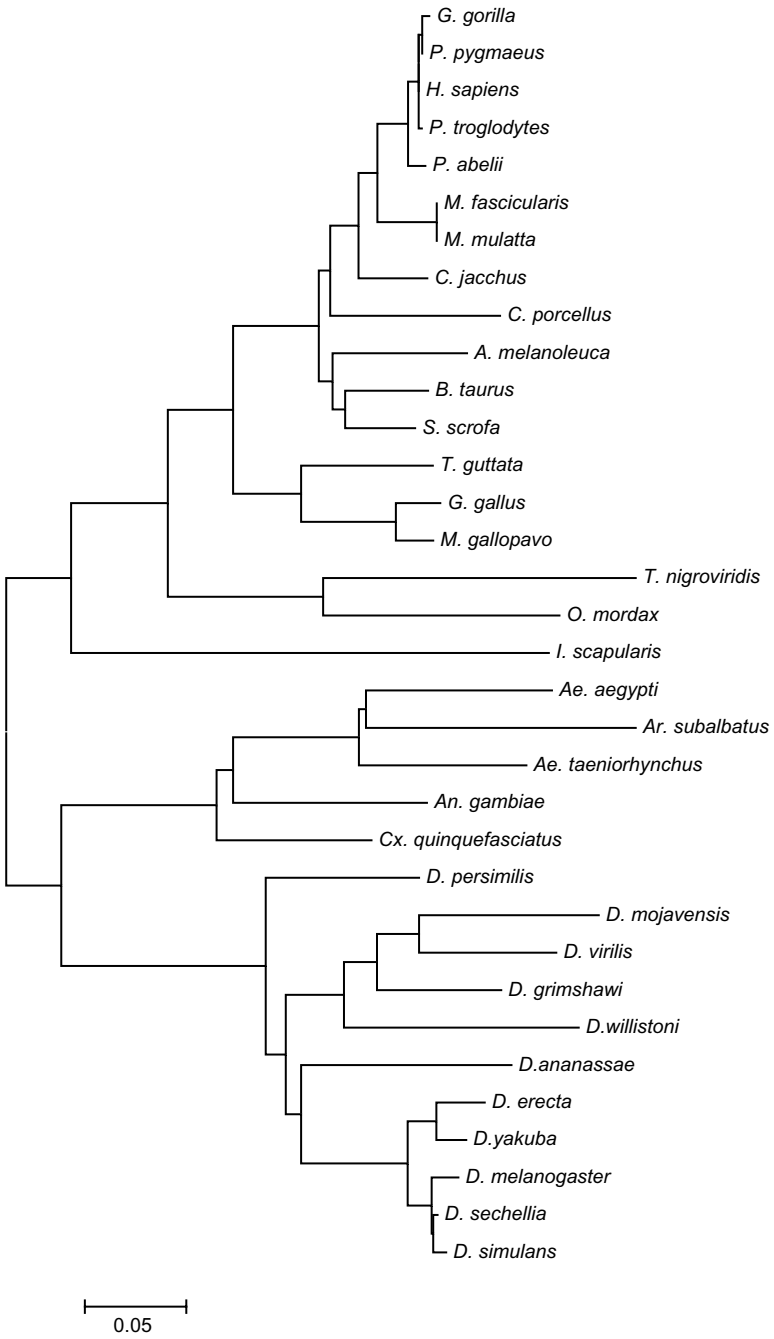

Figure 1 A phylogenetic tree for *NDUFS8* orthologs.

Notes: The phylogenetic tree was constructed using the Neighbor-joining tree-making method for NDUFS8 nucleic acid sequences of NADH dehydrogenase-ubiquinone Fe-S protein 8 subunit from other orthologues using the MEGA 5.05 program. The scale bar indicates the number of changes inferred as having occurred along each branch. The accession number of nucleic acid sequences of *NDUFS8* orthologues used in this analysis are FJ458415.1, AY432654.1, AY440457.1, XM_001868794, XM-321378.5, XM_0021032227.1, XM_002030974, NM_0799980.3, XM_001980072.1, XM_002097653, XM_001990316, XM_002016993, XM_001954946, XM_002056337.1, XM_001998624.1, XM_002074083.1, BX933502.2, XM_002755626.1, XM_002400792, NM_002496.3, NM_0011131881.1, XM_003468313, BT075206.1, CR711559.2, XM_0031222435.1, XM_002921294.1, DQ885653.1, NM_001071780.1, M58717.1, DQ885652.1, XM_001104103.2, AB125184.1, XM_003206148.1, and XM_002196863.1.

mRNA expression at 1 hour postexposure compared with the control treatment (Figure 3, Tables S4 and S5). Permethrin/acetone HD-treated *Ae. taeniorhynchus* adults showed a decrease in *AetNDUFS8* mRNA expression at 3 hours postexposure, but permethrin/acetone LD-treated *Ae. taeniorhynchus* adults showed an increase in *AetNDUFS8* RNA expression at 3 hours postexposure compared with treatment with acetone only. The qPCR data showed that *AetNDUFS8* RNA expression levels after 6 hours treated with acetone only (control) were significantly increased in the 5-day female *Ae. taeniorhynchus,*

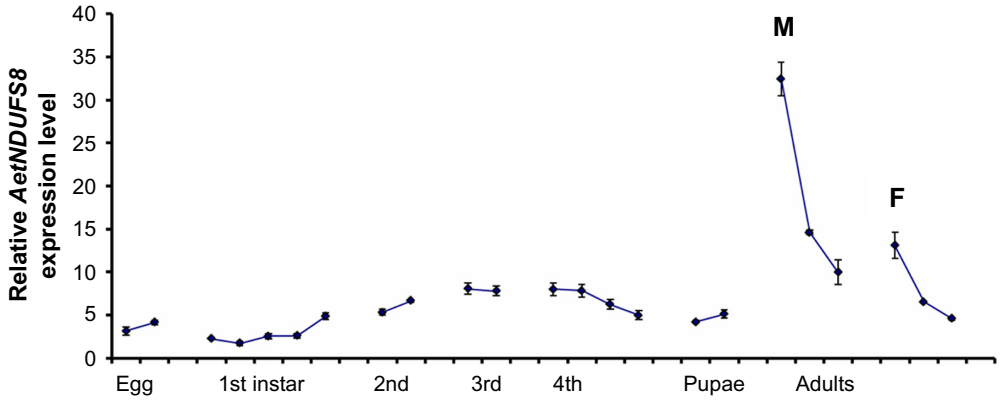

Figure 2 *AetNDUFS8* mRNA expression levels in eggs, larvae, pupae, and adults quantified by qPCR, with SD for three replicates.
Notes: Ages of eggs, 1 d, and 3 d, respectively; First instar, 5, 21, 29, 44, and 53 h posthatch, respectively; second instar, 69, and 77 h posthatch respectively; third instar, 93, and 101 h posthatch, respectively; fourth instar, 117, 125, 141, and 149 h posthatch, respectively; pupae, 165, and 173 h posthatch, respectively; and adults, and 1 d old male, (M) designated male, (ie, 8 d posthatch); 5 d old male (ie, 13 d posthatch); and 10 d old male (ie, 18 d posthatch); and 1 d old female, (F) designated female (ie, 8 d posthatch); 5 d old female (ie,13 d posthatch); and 10 d old female (ie, 18 d posthatch).
Abbreviations: d, day; h, hours; mRNA, messenger RNA, qPCR, quantitative real-time polymerase chain reaction; SD, standard deviation.

and then decreased after 24 hours of being treated with acetone only (Figure 3, Table S4).

AetNADH5 dehydrogenase gene expression in all developmental stages of *Aedes taeniorhynchus*

To understand the developmental regulation of mitochondrial encoded NADH dehydrogenase transcript under different physiological conditions, qPCR was performed to examine *AetNADH5* relative transcription levels in eggs, larvae, pupae, and adults (Figure 4, Table S6). The relative transcription level of the *AetNADH5* was a hundred to a thousand-fold higher than that of nuclear encoded *AetNDUFS8*. In the egg stage the relative mRNA transcription level of *AetNADH5* was highly expressed and slightly decreased over time from day 1 (1364.1±146.9) to day 3 (1278.1±35.84). Compared with the egg stages, *AetNADH5* mRNA expression was significantly reduced in the 1st instar larvae (116.28±5.16) (Figure 4, Tables S3, S7, and S8). The RNA relative expression level of *AetNADH5* was slightly increased through the development of the 1st instar larva in the early (21 hours posthatch) to the late (53 hours posthatch) stage samples. Compared with the 1st instar larvae, *AetNADH5* expression was relatively high in the 2nd instar larvae, the 3rd instar larvae, and the 4th instar larvae examined (from 69 hours posthatch to 125 hours posthatch) (Figure 4, Tables S6 and S7). However, RNA relative expression level of *AetNADH5*

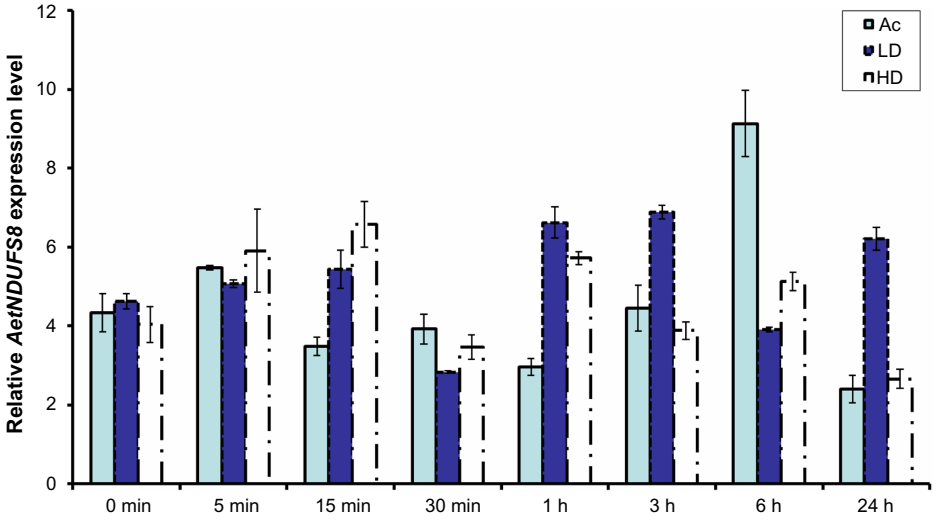

Figure 3 *AetNDUFS8* mRNA expression levels in 5 d old female treated topically with permethrin/acetone at 2.5×10^{-5} µg (high dose, HD), and 1.25×10^{-5} µg (low dose, LD) per mosquito, and acetone (Ac) quantified by qPCR, with standard deviation (SD) for three replicates. Please note X-axis is not to scale. Five-day-old adult postexposure to permethrin at 0, 5, 15, 30, 60, 180, and 360 min, and 24 h.
Abbreviations: d, day; h, hours; min, minutes; mRNA, messenger RNA; qPCR, quantitative real-time polymerase chain reaction.

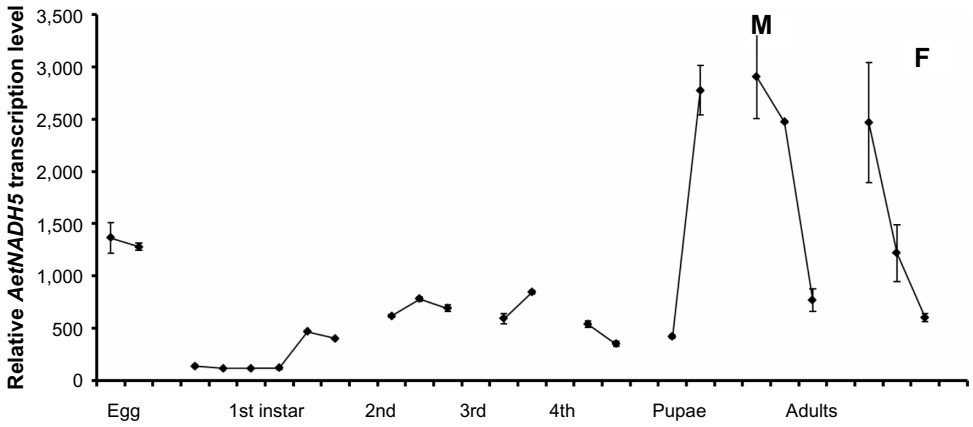

Figure 4 *AetNADH5* mRNA expression levels in eggs, larvae, pupae, and adults quantified by qPCR, with SD for three replicates.
Notes: Ages of eggs, 1 d, and 3 d, respectively; First instar, 5, 21, 29, 44, and 53 h posthatch, respectively; second instar, 69, and 77 h posthatch respectively; third instar, 93, and 101 h posthatch, respectively; fourth instar, 117, 125, 141, and 149 h posthatch, respectively; pupae, 165, and 173 h posthatch, respectively; and adults, and 1 d old male, (M) designated male, (ie, 8 d posthatch); 5 d old male (ie, 13 d posthatch); and 10 d old male (ie, 18 d posthatch); and 1 d old female, (F) designated female (ie, 8 d posthatch); 5 d old female (ie,13 d posthatch); and 10 d old female (ie, 18 d posthatch).
Abbreviations: d, day; h, hours; mRNA, messenger RNA; qPCR, quantitative real-time polymerase chain reaction; SD, standard deviation.

increased significantly between early pupae (422.33±19.69) and late pupae (2,775.9±1.469) mosquitoes. RNA relative expression level of *AetNADH5* remained high between teneral male (2,903.4±398.4) and female (2,467.0±575.6) mosquitoes (Figure 4, Table S6). RNA expression of *AetNADH5* in teneral adult *Ae. taeniorhynchus* was significantly higher than that found in 5- and 10-day-old adults (Figure 4, Tables S6, and S7).

Discussion

AetNDUFS8 transcription during *Ae. taeniorhynchus* development

The *AetNDUFS8* mRNA transcriptions in *Ae. taeniorhynchus* eggs, larvae, pupae, and adults have been analyzed using qPCR. The *AetNDUFS8* mRNA is expressed at low levels in eggs and the early larval stages and is expressed in varying quantities during the late larval stages and pupae. *AetNDUFS8* mRNA transcription varied in females and males of different ages. There were significant differences in the transcription of *AetNDUFS8* between teneral and 10-day-old *Ae. taeniorhynchus* females and males (Figure 2). In males, *AetNDUFS8* mRNA expression is higher than in females for all ages examined. In addition, *AetNDUFS8* expression was significantly different between teneral males and females (Figure 2), which may suggest that *AetNDUFS8* plays an important and different role depending on adult *Ae. taeniorhynchus* sex and age. *AetNDUFS8* gene expression in mature mosquitoes is crucial for mitochondrial functions and may also be related to mosquito aging. Furthermore, the relatively lower levels of *AetNDUFS8* transcription in older or senescent females and males suggests that mitochondrial dysfunction may also play a role in the attenuation of gene expression, which is similar to the *cytochrome C* gene expression in *Ae. aegypti*.[32]

Effect of permethrin on *AetNDUFS8* mRNA expression

Over the past decade, molecular studies of insecticide resistance have advanced rapidly. Many genes involved in target site and metabolic resistance mechanisms have been identified. The transcriptional regulation of gene expression is a primary approach by which insects adapt to a changing environment.[32] The evolution of insecticide resistance acts by selection of these mechanisms, typically requiring the interaction of multiple genes.[32] It is also reported that pesticides affected the interaction of multiple genes in mosquitoes.[32,33] In the other study, the expression of ~1/4 of the detoxification genes in *An. gambiae* was found to be developmentally regulated.[34] One of the best-known organic pesticides is Rotenone, an inhibitor of Complex I, which is found in several genera of tropical leguminosae plants.[35] Several hydrophobic and amphipathic compounds including some detergents inhibit the ubiquinone reductase reaction of respiratory chain Complex I.[36]

AetNDUFS8 mRNA expression levels of 5-day old adults of *Ae. taeniorhynchus* were significantly upregulated at 15 minutes after permethrin treatments (both HD and LD). During the time course study, *AetNDUFS8* mRNA expression level of 5-day old female adults of *Ae. taeniorhynchus* showed unpredictable fluctuation between HD and LD permethrin treatments compared with the control. This indicated that *AetNDUFS8* transcript levels in adult *Ae. taeniorhynchus* might have differences in response to the different concentration of permethrin treatment, which might

reveal the pesticide mechanism in the mosquito related role of the NADH in the respiratory function of Complex I. Furthermore, understanding of the pesticide mechanism in the mosquito may help to find new inhibitors of Complex I, which could be used as new pesticides.[21]

Mitochondrially encoded *AetNADH5* transcription during *Ae. taeniorhynchus* development

In the current study, we also demonstrated the expression of *AetNADH5* in different developmental stages of *Ae. taeniorhynchus* by qPCR. Our results revealed that *AetNADH5* was expressed throughout the developmental stages of *Ae. taeniorhynchus*. However, the expression level of *AetNADH5* in embryonic stage was significantly higher than that in the larval stages (Figure 3). This observation is of importance in that it suggests that *AetNADH5* might play a functional role in embryonic development in *Ae. taeniorhynchus*. Our results also revealed that *AetNADH5* expression levels in late pupal and early adult stages were significantly higher than in the larval stage. Furthermore, at different times within each developmental stage, the expression levels of *AetNADH5* were different, suggesting that *AetNADH5* plays a critical role throughout the physiological process of the development of *Ae. taeniorhynchus*. Meanwhile, the relative low levels of *AetNADH* transcription in older or senescent males and females suggest that mitochondrial dysfunction may also play a role in the attenuation of gene expression, which is similar to the *cytochrome c* and *cytochrome b* gene expression in the *Ae. aegypti*.[26,27]

The crucial role of NADH in the respiratory function of Complex I has been demonstrated by linking mutation in subunits to certain diseases.[14] In humans, mutations in the subunits of Complex I can cause mitochondrial diseases, including Leigh syndrome known as Subacute Necrotizing Encephalomyelopathy.[37–40] Mitochondrial dysfunction has been associated with Parkinson's disease, particularly widely demonstrated in Complex I impairment and subsequent oxidative stress.[41–45] There are also many mitochondrial dysfunction mutations in insects, such as *Drosophila*.[46–50]

The mitochondrial gene expression data can be used in RNAi studies to investigate the functional role of mitochondrial genes in mosquito development. The mitochondrial gene NADH can also provide information to identify ecotypes of mosquitoes from different geographical locations in the United States and other countries.[9]

In conclusion, the expressions of *AetNDUFS8* and *AetNADH5* in the life cycle of *Ae. taeniorhynchus* were regulated developmentally and environmentally. The mRNA expressions of *AetNDUFS8* and *AetNADH5* have, for the first time, been examined in detail for all developmental stages of *Ae. taeniorhynchus*. This study suggests that *AetNDUFS8* and *AetNADH5* play an important role in the development of *Ae. taeniorhynchus* and will provide information useful for designing dsRNA pesticide for mosquito control.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Expression of *AetNDUFS8* in different developmental stages of *Aedes taeniorhynchus*

Sample stage	Sample name	Sample time	Mean cycle threshold (Ct) ± SD		Relative *AetNDUFS8* expression level			
			rR40s	*AetNDUFS8*	ΔCt–1	ΔCt–2	ΔCt–3	$100\times2^{-\Delta Ct}$ ± SD
Eggs	Egg 1	1 d	14.089±0.041	19.156±0.045	5.064	5.069	5.067	2.984±0.071
	Egg 2	3 d	14.840±0.013	19.509±0.039	4.669	4.649	4.688	3.931±0.074
1st instar larvae	Larvae 1	5 h[a]	11.588±0.209	17.044±0.127	5.398	5.515	5.462	2.279±0.132
	Larvae 2	21 h[a]	10.852±0.101	16.585±0.143	5.704	5.763	5.733	1.880±0.054
	Larvae 3	29 h[a]	12.815±0.182	18.121±0.038	5.461	5.149	5.305	2.544±0.386
	Larvae 4	44 h[a]	12.815±0.182	17.532±0.057	5.093	5.242	5.167	2.787±0.203
	Larvae 5	53 h[a]	13.060±0.115	17.326±0.023	4.331	4.199	4.265	5.205±0.334
2nd instar larvae	Larvae 6	69 h[a]	12.933±0.012	17.211±0.111	4.192	4.365	4.278	5.162±0.439
	Larvae 7	77 h[a]	13.100±0.055	17.310±0.036	3.919	3.939	3.929	6.564±0.062
3rd instar larvae	Larvae 8	93 h[a]	12.339±0.034	16.054±0.005	3.742	3.726	3.688	7.617±0.202
	Larvae 9	101 h[a]	12.427±0.021	16.148±0.013	3.725	3.715	3.720	7.586±0.039
4th instar larvae	Larvae 10	117 h[a]	12.386±0.034	16.131±0.033	3.747	3.742	3.744	7.459±0.015
	Larvae 11	125 h[a]	13.228±0.060	17.005±0.077	3.789	3.765	3.777	7.295±0.086
	Larvae 12	141 h[a]	13.845±0.064	17.803±0.129	3.821	4.095	3.958	6.464±0.864
	Larvae 13	149 h[a]	14.210±0.007	15.316±0.044	4.211	4.216	4.214	5.390±0.013
Pupae	P1	165 h[a]	14.191±0.020	18.717±0.011	4.518	4.534	4.526	4.341±0.034
	P3	173 h[a]	15.216±0.026	19.568±0.043	4.401	4.303	4.352	4.901±0.235
Adults	A1 (M)[b]	1 d	15.887±0.021	17.480±0.048	1.545	1.642	1.593	33.158±1.573
	A2 (M)[b]	5 d	18.641±0.076	21.423±0.034	2.811	2.751	2.781	14.549±0.426
	A3 (M)[b]	10 d	17.961±0.055	21.257±0.190	3.200	3.391	3.296	10.204±0.954
	A1(F)[c]	1 d	15.787±0.073	18.637±0.226	2.959	2.742	2.850	13.904±1.469
	A2 (F)[c]	5 d	15.273±0.026	19.226±0.006	3.976	3.931	3.953	6.4571±0.143
	A3 (F)[c]	10 d	15.987±0.039	20.389±0.046	4.397	4.407	4.402	4.729±0.023

Notes: [a]Hours post hatch; [b]males; [c]females.
Abbreviations: d, day; F, female; M, male; SD, standard deviation.

Table S2 Paired *t*-test data for comparison of relative *AetNDUFS8* gene transcription between female (F) and male (M), as well as different ages between the same sex (either female or male) in *Aedes taeniorhynchus*

Sexes and ages	N	*df*	*t*-value	*P*-value
F1-d and M1-d	3	2	−15.841	0.004*
F5-d and M5-d	3	2	−70.216	<0.001*
F10-d and M10-d	3	2	−14.410	0.005*
F1-d and F5-d	3	2	14.583	0.005*
F1-d and F10-d	3	2	15.851	0.004*
M1-d and M5-d	3	2	22.810	0.002*
M1-d and M10-d	3	2	90.879	<0.001*

Note: *Statistical significance ($P<0.05$).
Abbreviations: d, day; *df*, degrees of freedom.

Table S3 Paired *t*-test data for comparison of relative *AetNDUFS8* gene transcription between eggs, larvae, pupae, and adults in *Aedes taeniorhynchus*

Stage and ages	N	*df*	*t*-value	*P*-value
Egg 1 and egg 3	3	2	−34.939	<0.001*
Egg 3 and larvae 1	3	2	−71.951	<0.001*
Larvae 1 and larvae 2	3	2	12.861	0.006*
Larvae 2 and larvae 3	3	2	3.691	0.066
Larvae 3 and larvae 4	3	2	−1.012	0.418
Larvae 4 and larvae 5	3	2	11.008	0.008*
Larvae 5 and larvae 6	3	2	0.136	0.904
Larvae 6 and larvae 7	3	2	9.026	0.012*
Larvae 7 and larvae 8	3	2	−9.897	0.010*
Larvae 8 and larvae 9	3	2	−0.462	0.689
Larvae 9 and larvae 10	3	2	12.522	0.006*
Larvae 10 and larvae 11	3	2	−5.675	0.030*
Larvae 11 and larvae 12	3	2	2.139	0.166
Larvae 12 and larvae 13	3	2	−3.088	0.091
Larvae 13 and pupae 1	3	2	120.587	<0.001*
Pupae 1 and pupae 2	3	2	5.095	0.036*
Pupae 2 and M1-d	3	2	−17.856	0.003*
Pupae 2 and F1-d	3	2	−38.296	<0.001*

Note: *Statistical significance ($P<0.05$).
Abbreviations: d, day; *df*, degrees of freedom, F, female; M, male.

Table S4 Expression of *AetNDUFS8* under permethrin stress conditions in *Aedes taeniorhynchus*

Time point	Mean cycle threshold (Ct) ± SD		Relative *AetNDUFS8* expression level			
	rR40s	*AetNDUFS8*	ΔCt–1	ΔCt–2	ΔCt–3	$100\times2^{-\Delta Ct}$ ± SD
Ac-0 min[a]	13.287±0.022	17.822±0.063	4.374	4.697	4.536	4.3117±0.685
Ac-5 min	13.687±0.064	17.879±0.040	4.175	4.209	4.191	5.4717±0.091
Ac-15 min	12.679±0.153	17.526±0.289	4.751	4.943	4.847	3.4826±0.326
Ac-30 min	12.647±0.077	17.325±0.120	4.538	4.817	4.678	3.9255±0.535
Ac-1 h	12.217±0.012	17.296±0.136	4.973	5.183	5.078	2.9678±0.305
Ac-3 h	12.693±0.290	17.191±0.025	4.686	4.311	4.498	4.4627±0.816
Ac-6 h	13.229±0.261	16.688±0.072	3.591	3.325	3.458	9.1385±1.186
Ac-24 h	11.654±0.120	17.045±0.194	5.176	5.605	5.390	2.4106±0.504
LD-0 min[b]	13.285±0.027	17.721±0.037	4.374	4.497	4.436	4.6212±0.280
LD-5 min	13.458±0.174	17.761±0.137	4.328	4.277	4.303	5.0686±0.127
LD-15 min	13.085±0.042	17.292±0.223	4.079	4.336	4.208	5.4341±0.681
LD-30 min	13.213±0.036	18.355±0.015	5.127	5.157	5.142	2.8318±0.042
LD-1 h	13.233±0.013	17.153±0.109	3.833	4.006	3.919	6.6212±0.561
LD-3 h	13.389±0.012	17.251±0.064	3.825	3.899	3.862	6.8820±0.249
LD-6 h	12.497±0.007	17.174±0.039	4.655	4.699	4.677	3.9098±0.086
LD-24 h	12.172±0.084	16.181±0.010	3.943	4.076	4.010	6.2151±0.404
HD-0 min[c]	13.086±0.119	17.721±0.047	4.473	4.797	4.636	4.0229±0.639
HD-5 min	11.385±0.237	15.483±0.127	4.356	3.840	4.098	5.9338±1.483
HD-15 min	12.164±0.086	16.093±0.093	3.802	4.056	3.929	6.5909±0.817
HD-30 min	12.098±0.099	16.956±0.082	4.730	4.987	4.858	3.4611±0.434
HD-1 h	12.621±0.071	16.749±0.012	4.087	4.170	4.128	5.7196±0.234
HD-3 h	12.120±0.091	16.809±0.023	4.769	4.609	4.689	3.8822±0.306
HD-6 h	12.770±0.175	17.057±0.081	4.354	4.221	4.287	5.1275±0.334
HD-24 h	12.639±0.025	17.875±0.221	5.105	5.369	5.237	2.6631±0.343

Notes: [a]Acetone treatments in 5 d old female *Ae. taeniorhynchus;* [b]pemethrin LD treatment in 5 d old female *Ae. taeniorhynchus;* [c]pemethrin HD treatment in 5 d old female *Ae. taeniorhynchus.*

Abbreviations: Ac, acetone; h, hours; min, minutes; SD, standard deviation; LD, low dose; HD, high dose.

Table S5 Paired *t*-test data for comparison of relative *AetNDUFS8* gene transcription between acetone, low dose (LD), and high dose (HD) permethrin treatments in *Aedes taeniorhynchus*

Permethrin treatments	N	*df*	*t*-value	*P*-value
LD and AC 0 min	3	2	−1.776	0.218
LD and AC 5 min	3	2	8.200	0.015*
LD and AC 15 min	3	2	−13.405	0.006*
LD and AC 30 min	3	2	11.273	0.008*
LD and AC 1 h	3	2	−34.620	<0.001*
LD and AC 3 h	3	2	−5.586	0.031*
LD and AC 6 h	3	2	10.039	0.010*
LD and AC 24 h	3	2	−94.119	<0.001*
HD and AC 0 min	3	2	−16.233	<0.004*
HD and AC 5 min	3	2	−0.666	0.574
HD and AC 15 min	3	2	−15.466	0.004*
HD and AC 30 min	3	2	−11.273	0.008*
HD and AC 1 h	3	2	−88.490	<0.001*
HD and AC 3 h	3	2	2.722	0.113
HD and AC 6 h	3	2	11.467	0.008*
HD and AC 24 h	3	2	−3.938	0.059
LD and HD 0 min	3	2	3.943	0.059
LD and HD 5 min	3	2	−1.515	0.269
LD and HD15 min	3	2	−20.865	0.002*
LD and HD 30 min	3	2	5.394	0.033*
LD and HD 1 h	3	2	6.700	0.022*
LD and HD 3 h	3	2	13.264	0.006*
LD and HD 6 h	3	2	−7.119	0.019*
LD and HD 24 h	3	2	141.984	<0.001*

Note: *Statistical significance ($P<0.05$).

Abbreviations: AC, acetone; *df*, degrees of freedom, h, hours; min, minutes.

Table S6 Expression of *AetNADH5* in different developmental stages of *Aedes taeniorhynchus*

Sample stage	Sample name	Sample time	Mean cycle threshold (Ct) ± SD		Relative *AetNADH5* expression level			
			rR40s	*AetNADH5*	ΔCt–1	ΔCt–2	ΔCt–3	$100\times2^{-\Delta Ct}$ ± SD
Eggs	Egg 1	1 day	12.849±0.101	9.079±0.054	–3.925	–3.615	–3.769	1364.1±146.9
	Egg 2	3 day	13.624±0.120	9.065±0.041	–3.716	–3.636	–3.676	1278.1±35.84
1st instar larvae	Larvae 1	5 h[a]	10.512±0.447	10.051±0.151	–0.310	–0.612	–0.461	137.66±14.43
	Larvae 2	21 h[a]	10.676±0.333	10.458±0.269	–0.154	–0.282	–0.218	116.28±5.156
	Larvae 3	29 h[a]	11.406±0.084	11.198±0.037	–0.293	–0.198	–0.245	118.52±3.911
	Larvae 4	44 h[a]	9.1631±0.908	8.8999±0.162	–0.101	–0.425	–0.263	120.01±13.53
	Larvae 5	53 h[a]	11.542±0.112	9.3146±0.092	–2.048	–2.027	–2.228	468.37±6.672
2nd instar larvae	Larvae 6	69 h[a]	11.375±0.098	9.3639±0.074	–1.988	–2.035	–2.011	403.18±6.582
	Larvae 7	77 h[a]	11.769±0.029	9.143±0.003	–2.660	–2.591	–2.626	617.16±14.74
3rd instar larvae	Larvae 8	93 h[a]	10.874±0.057	7.909±0.099	–3.006	–2.924	–2.965	780.74±22.32
	Larvae 9	101 h[a]	10.719±0.388	7.929±0.065	–2.856	–2.724	–2.789	691.51±31.63
4th instar larvae	Larvae 10	117 h[a]	10.245±0.259	7.675±0.118	–2.451	–2.687	–2.569	593.58±48.66
	Larvae 11	125 h[a]	11.198±0.034	8.122±0.027	–3.045	–3.101	–3.076	843.54±18.27
	Larvae 12	141 h[a]	12.446±0.069	10.019±0.018	–2.514	–2.339	–2.427	537.61±32.46
	Larvae 13	149 h[a]	12.835±0.092	11.022±0.008	–1.913	–1.712	–1.813	351.25±24.49
Pupae	P1	165 h[a]	13.134±0.003	11.055±0.069	–2.011	–2.146	–2.078	422.33±19.69
	P3	173 h[a]	13.962±0.124	9.167±0.012	–4.658	–4.931	–4.795	2775.9±236.1
Adults	A1 (M)[b]	1 day	12.939±0.073	8.079±0.197	–5.056	–4.661	–4.859	2903.4±398.4
	A2 (M)[b]	5 day	16.684±0.019	12.056±0.013	–4.634	–4.622	–4.628	2472.7±10.71
	A3 (M)[b]	10 day	15.954±0.045	13.012±0.196	–3.138	–2.746	–2.942	768.64±104.9
	A 1(F)[c]	1 day	13.845±0.027	9.221±0.359	–4.958	–4.292	–4.625	2467.0±575.6
	A1 (F)[c]	5 day	14.752±0.538	11.145±0.083	–3.690	–2.985	–3.607	1218.4±269.8
	A2 (F)[c]	10 day	13.838±0.006	11.252±0.095	–2.674	–2.497	–2.587	600.26±37.06

Notes: [a]hours post hatch; [b]males; [c]females.
Abbreviations: h, hours; F, female; M, male; SD, standard deviation.

Table S7 Paired *t*-test data for comparison of relative *AetNADH5* gene transcription between female (F) and male (M), as well as different ages between the same sex (either female or male) in *Aedes taeniorhynchus*

Sexes and ages	N	*df*	*t*-value	*P*-value
F1-d and M1-d	3	2	–3.989	0.057
F5-d and M5-d	3	2	–8.497	<0.014*
F10-d and M10-d	3	2	–4.399	0.048*
F1-d and F5-d	3	2	6.908	0.020*
F1-d and F10-d	3	2	6.142	0.025*
M1-d and M5-d	3	2	1.999	0.184
M1-d and M10-d	3	2	12.669	0.006*

Note: *Statistical significance ($P<0.05$).
Abbreviations: d, days; *df*, degrees of freedom.

Table S8 Paired *t*-test data for comparison of relative *AetNADH5* gene transcription between eggs, larvae, pupae, and adults in *Aedes taeniorhynchus*

Stage and ages	N	*df*	*t*-value	*P*-value
Egg 1 and egg 3	3	2	–1.175	0.361
Egg 3 and larvae1	3	2	39.295	<0.001*
Larvae 1 and larvae 2	3	2	3.276	<0.022*
Larvae 2 and larvae 3	3	2	6.000	0.438
Larvae 3 and larvae 4	3	2	–2.242	0.075
Larvae 4 and larvae 5	3	2	12.000	0.844
Larvae 5 and larvae 6	3	2	–2.242	0.031*
Larvae 6 and larvae 7	3	2	–28.819	0.001*
Larvae 7 and larvae 8	3	2	14.259	0.005*
Larvae 8 and larvae 9	3	2	5.385	0.033*
Larvae 9 and larvae 10	3	2	2.394	0.139
Larvae 10 and larvae 11	3	2	–14.178	0.005*
Larvae 11 and larvae 12	3	2	10.427	0.009*
Larvae 12 and larvae 13	3	2	40.517	<0.001*
Larvae 13 and pupae 1	3	2	–2.776	0.109
Pupae 1 and pupae 2	3	2	–28.885	0.001*
Pupae 2 and M1-d	3	2	–0.526	0.652
Pupae 2 and F1-d	3	2	0.933	0.449

Note: *Statistical significance ($P<0.05$).
Abbreviations: d, day; *df*, degrees of freedom.